

EFFECT OF LOW DOSES IONIZING RADIATION ON GENOMIC INSTABILITY AFTER VERTEBROPLASTY IN PATIENTS WITH OSTEOPOROSIS

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Abstract. Objective: Osteoporotic vertebral fractures affect 1.4 million patients in the world annually. Symptomatic fractures are treated with minimally invasive fluoroscopically guided medical procedures and, as a result, there is a considerable amount of low dose irradiation registered for patients and staff. According to the Linear no threshold model (LNT) used for evaluating radiation cancer risk, no radiation dose is considered safe. **Aim:** Our aim was to determine the amount of DNA damage after low dose irradiation of patients with osteoporosis undergoing vertebroplasty procedure. **Methods:** We used fluorescent microscope visualization of the immunochemical co-localization of γ -H2AX and 53BP1 DNA damage repair proteins, the so-called DNA damage-repair foci (DRF), to quantify the amount of DSBs (double-strand breaks) in lymphocytes from peripheral. The analysis included 42 patients within the age range of 60 ÷ 91 years old (71, mean), who received mean cumulative air kerma dose 22.07 mGy (5 ÷ 86.1 mGy, range). All doses received by the patients during fluoroscopy time were in the low dose range (≤ 100 mGy). **Results:** A total of 8,200 cells were analyzed. We found a statistically significant increase in γ -H2AX/53BP1 DRF frequency in patients after vertebroplasty 1.28 ± 0.06 compared to the initial frequency before the procedure 0.70 ± 0.04 ($p < 0.0001$). This rise in DRF was not statistically correlated with the amount of cumulative air kerma dose received nor the age of the patients. **Conclusion:** This preliminary study showed a significant DNA damage increase after minimally invasive vertebroplasty procedures despite the low and very low doses received during fluoroscopy time. Miss-repaired DNA DSBs have the potential to manifest in stable chromosomal aberrations.

Key words: vertebroplasty, fluoroscopy, osteoporosis, ionizing radiation, low doses, very low doses, genomic instability, DNA double-strand breaks, γ -H2AX/53BP1 foci

INTRODUCTION

According to the estimations, osteoporotic vertebral fractures affect 1.4 million patients in the world annually [1]. In Europe, 400,000 new vertebral fractures are diagnosed yearly and, according to the demographic prognostics, this number may be doubled by the year 2050 [2-4]. Osteoporosis is responsible for 80% of all vertebral fractures and recently the World Health Organization (WHO) has recognized this disease as emerging health problem in aging societies [5]. Although most patients have mild symptoms, a subset of patients develop substantial pain and disability and some require admission to hospital. Standard therapy, consisting of rest, analgesia, and immobilization, is often poorly tolerated in elderly people [6, 7].

Increasing use of minimally invasive surgical solutions (MISS) allows prediction of the growing number of patients treated with these modalities in the future. These techniques as compared with open surgery offer such advantages as smaller wounds, negligible blood loss, shorter hospitalization time, and lower risk of complications. However, the risk of radiation for pa-

tients and medical staff is the main drawback of MISS procedures, which are generally performed under fluoroscopic guidance [8]. Vertebroplasty, the injection of polymethylmethacrylate (PMMA) into the fractured vertebral body, is frequently used for symptomatic osteoporotic fractures, and is based on the premise that fracture stabilization can provide pain relief. Fluoroscopy is used during this procedure for obtaining real time X-ray imaging [7, 9].

The effects of low-dose irradiation on human health have not been definitively elucidated, and the efforts of professionals and the public regarding the safe and effective use of radiation in medicine are focused on the radiation protection of the patient. The International Atomic Energy Agency (IAEA) developed educational, reporting and information systems in the field of radiation protection for interventional procedures under fluoroscopic control (SAFRAD) [10]. Doses received in interventional radiology cause significant DNA damage in the blood cells of patients [11, 12] and medical personnel [13].

Ionizing radiation is a versatile mutagen, initiating a wide range of DNA double-strand breaks (DSBs).

If cell death does not occur, the risk of developing neoplasms may increase due to the accumulation of cells with miss-repaired DNA and the consequent genetic instability [14, 15]. It is established that the formation of DSBs correlates with the radiation dose [16]. IR-induced DNA DSBs lead to the phosphorylation of a highly conserved serine 139 on the histone H2AX isoform and can be detected within 3 min after exposure [17]. To assess quantitatively the DNA radiation damage on the peripheral blood lymphocytes, an immunochemical co-localization of γ -H2AX and 53BP1 proteins is widely used. As a result, in the cells the so-called DNA damage-repair foci (DRF) are formed and analyzed with the use of fluorescence microscopy. DRF are a valuable marker in low-dose biodosimetry and their appearance directly correlates with the count of DNA DSBs, the sensitivity of the method being limited to 0.003 Gy [18-20].

The purpose of this study was to determine exemplarily the amount of DNA damage after interventional radiology by using visualization of γ -H2AX foci to quantify DNA DSBs at vertebroplasty. At present to our knowledge there is no scientific literature on analyzes of the effect of low doses of ionizing radiation on vertebroplasty patients and accumulating data on genetic alterations from low-dose medical irradiation will contribute to the current risk assessment of these spinal interventions.

MATERIALS AND METHODS

Blood Samples

In this study, 42 vertebroplasty patients with osteoporosis were enrolled. Two peripheral blood samples were taken from each patient immediately before and after the medical procedure. For every patient the following data were obtained: age, sex, weight, height, length of fluoroscopy time, number of levels of the vertebrae operated and cumulative air kerma dose received as recorded by the Philips BV Pulsera X-ray apparatus used during the medical procedure. Population group of healthy individuals was used as control group.

γ -H2AX/53BP1 foci immunofluorescence staining and microscope analyses

Peripheral blood from all patients was drawn by venipuncture and collected in tubes with Heparin (Greiner-Bio-One). Cells were isolated from the blood samples using Histopaque 1077 (Sigma-Aldrich), according to the manufacturer's protocol. The cells were washed three times in phosphate buffered saline (PBS), resuspended and counted automatically

(Countess™, Invitrogen). Their viability was > 85%, estimated by trypan blue dye exclusion assay. Isolated cells were then spotted onto microscope slides (Thermo Scientific) at a concentration of $\sim 1 \times 10^6$ cells/ml. Fixation followed in 2% formaldehyde (Polysciences Incorporated), permeabilization in 0.25% Triton X-100 (Sigma-Aldrich) and blocking with 1% BSA/PBS (Sigma-Aldrich). Incubation was applied with two primary antibodies: Mouse anti-phospho-H2AX Ser139 (Millipore, Cat # 05-636-I) and Rabbit polyclonal to 53BP1 (Abcam, Cat # ab36823), diluted to 1:500 and 1:400, accordingly, in 1% BSA/PBS. After 40 min of incubation, the pellet was washed with 1% BSA/PBS and the following secondary antibodies were added: Alexa Fluor 488 goat anti-mouse (Invitrogen, Cat # A11029) and Alexa Fluor 555 goat anti-rabbit (Invitrogen, Cat # A21429) diluted to 1:500 in 1% BSA/PBS. After 20 min of incubation (the samples were stored in dark), the pellet was washed with 1% BSA/PBS. The slides were then dried, mounted with DAPI Vectashield solution (Vector Laboratories) and stored at -20°C for further analysis. Microscope slides were analyzed using Olympus BX41 fluorescence microscope (equipped with filter blocks for excitation of red, green and blue fluorescence). 100 cells were analyzed for each patient and for each experimental point (before and after the procedure). All counting was performed manually using a 100 x immersion objective.

Statistical analyses

The frequency of γ -H2AX/53BP1 DNA damage-repair foci (DRF) was estimated using the formula $\Sigma DRF/n$ cells, where ΣDRF is the total number of DRF in the scored cells and n cells is the number of cells analyzed. Research data obtained were analyzed with MS Excel, SigmaPlot 14.5 and IBM SPSS Statistics 23. A t-test, paired t-test, Pearson correlation test and Multiple linear regression tests were used for normally distributed data. Continuous variables were expressed as *mean $\pm$ standard error of mean (SEM)*.

RESULTS

Main characteristics of the patients group

The analysis included 42 patients with osteoporosis undergoing vertebroplasty procedure. 74% of the subjects were females and 26% male individuals within the age range of $60 \div 91$ years old (71, mean). All subjects had osteoporosis and received trauma of the vertebrae. 52% of the traumas were lumbar and 48% thoracic. Three of the patients had lumbar and thoracic traumas combined. 26 of the subjects received treatment on 1 vertebrae lev-

el, 13 on 2 levels, 2 of the patients on 3 levels and one subject received medical treatment on 4 lumbar levels. Mean fluoroscopy procedure time was 1:15 min ($0:12 \div 2:53$, range) with mean cumulative air kerma dose 22.07 mGy ($5 \div 86.1$, range) as recorded by the X-ray apparatus used during the medical intervention. The baseline demographic, clinical and laboratory features of the study population, as well as the parameters measuring the fluoroscopy x-ray exposure, are listed in Table 1.

Table 1. The characteristics of vertebroplasty group

Patients, n	42
Male/female, %	26/74
Mean (range) age, y	71(60÷91)
BMI	26.0(20.2÷35.5)
Etiology, %	
Osteoporosis	100
Trauma	100
Anatomic location, %	
Lumbar	52
Thoracic	48
Level treated, n patients	
1	26
2	13
3+	3
Mean fluoroscopy procedure time (range)min	1:15(0:12÷2:53)
Mean irradiation dose(range), mGy	22.07(5÷86.1)

Frequency of γ -H2AX/53BP1 DNA damage-repair foci (DRF) analyses

A total of 8,200 cells were analyzed for γ -H2AX/53BP1 DNA damage-repair foci formation using fluorescence labeling before and after the procedure. Exemplary microscope images of co-localized DRF are presented in Fig. 1.

The spontaneous frequency of γ -H2AX/53BP1 DNA damage-repair foci in peripheral blood lymphocytes for a Bulgarian population was 0.29 ± 0.08 . The frequency of DRF in vertebroplasty patients

were 0.70 ± 0.04 before and 1.28 ± 0.06 after the medical procedure. There was statistically significant increase in the frequency of γ -H2AX/53BP1 DNA damage-repair foci in patients after vertebroplasty ($p < 0.001$). In addition, there was a statistically significant difference in the frequency of DRF in patients before vertebroplasty compared to the population control group ($p < 0.001$). (Fig. 2 and Fig. 3). The mean increase in the frequency of DRF in patients was $205.51\% \pm 12.40$ after vertebroplasty – minimum increase was 8% and maximum increase was 422.86% after the medical procedure. For one patient only a decrease in the frequency of DRF was recorded: -11.83%.

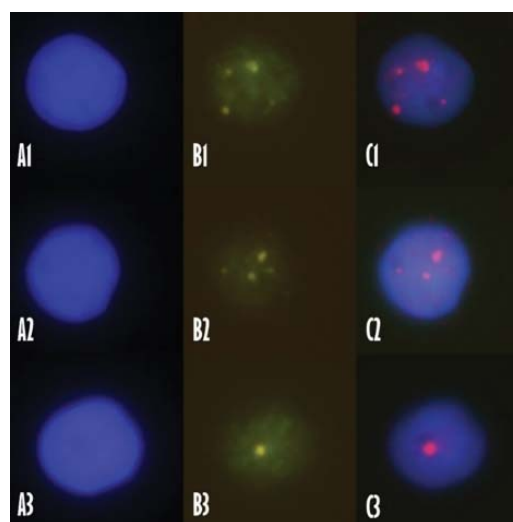


Fig. 1. Microscope images of co-localized γ -H2AX/53BP1 DNA damage-repair foci in a patient. Visualization of nuclear DAPI staining in blue (A), 53BP1 protein in green (B) and γ -H2AX protein in red colorization

For further analyses, the patients were separated in four dose groups. First group – patients who received cumulative air kerma dose up to 10 mGy, second group – 11-20 mGy, third group – 21-30 mGy,

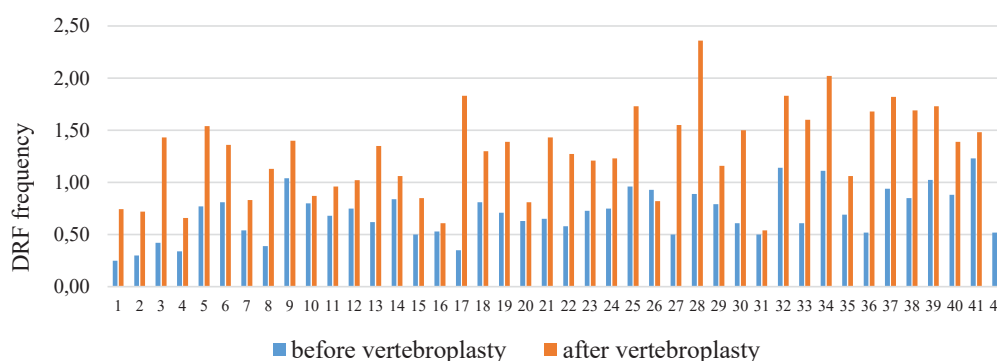


Fig. 2. Frequency of γ -H2AX/53BP1 DNA damage-repair foci in peripheral blood cells in 42 vertebroplasty patients before and after the medical procedure

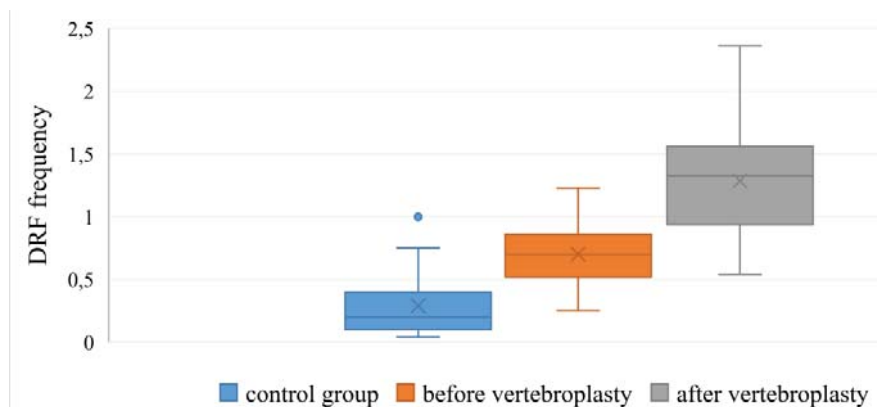


Fig. 3. Frequency of γ -H2AX/53BP1 DNA damage-repair foci in peripheral blood lymphocytes in control group of the population and in patients before and after vertebroplasty

Table 2. The characteristics of vertebroplasty patients in four dose groups

Cumulative air kerma dose [mGy]	number of patients [N]	frequency of DRF before vertebroplasty ($\pm$ SEM)	frequency of DRF after vertebroplasty ($\pm$ SEM)
0,1 - 10	14	0.80 ($\pm$ 0.07)	1.37 ($\pm$ 0.10)*
11-20	9	0.62 ($\pm$ 0.06)	1.3 ($\pm$ 0.16)*
21-30	12	0.68 ($\pm$ 0.05)	1.21 ($\pm$ 0.14)**
31+	7	0.65 ($\pm$ 0.10)	1.22 ($\pm$ 0.15)***

* $p < 0.001$; ** $p = 0.002$; *** $p = 0.009$

and fourth group – the patients who received more than 31 mGy during vertebroplasty. For each dose group, there was a statistically significant increase in the frequency of DRF after vertebroplasty compared to the frequency before the medical procedure. No statistically significant difference was found in the incidence of DRF after vertebroplasty between the four dose groups (Table 2).

Linear regression analyses showed no correlation between participants age and the frequency of γ -H2AX/53BP1 DNA damage-repair foci after vertebroplasty ($p > 0.05$).

Dose and BMI correlation analyses

A multiple linear regression model showed dependency in cumulative air kerma dose and the body mass index (BMI) of vertebroplasty patients; this dependency was accounted for the number of levels of the vertebrae operated. There was a tendency of increasing the dose with increasing BMI of the patient ($p < 0.05$) (Fig. 4).

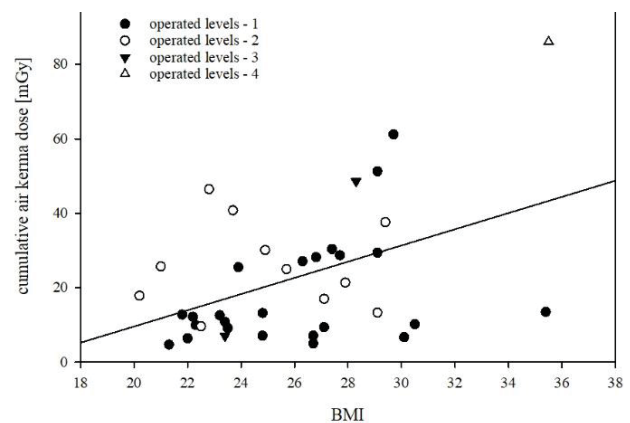


Fig. 4. A trend of increasing the cumulative air kerma dose received after vertebroplasty with increasing the patients BMI, accounted for the number of vertebrae levels operated

DISCUSSION

In the present study, we used γ -H2AX/53BP1 DNA damage-repair foci (DRF) as a biomarker for

radiation-induced DNA double-strand breaks (DSB) and to assess the potential genotoxicity of low doses of ionizing radiation in peripheral blood lymphocytes of 42 patients with osteoporosis undergoing vertebroplasty. In all patients enrolled in the study, there was a tendency of increase of the frequency of DRF after vertebroplasty compared to the initial frequency before the medical procedure.

Our findings are in line with other scientific research on the effects of low dose of ionizing radiation. An increase in the frequency of γ -H2AX DNA damage-repair foci and an increased incidence of DNA damage in lymphocytes of patients has been noted with low doses of radiation received during medical radiological procedures [21, 22]. Jin et al. [23] reported a statistically significant increase in the frequency of γ -H2AX DRF in mononuclear cells in patients undergoing cardiac catheterizations. Elevated levels of γ -H2AX DRF have also been found after pediatric cardiac catheterizations, after CT examinations, and after percutaneous transluminal angioplasty [24, 25].

Our analyses suggested inter-individual variability in the induction of γ -H2AX/53BP1 DNA damage-repair foci after irradiation with low and very low doses. In one patient, there was 8% increase in the frequency of DRF compared to another patient with 422.86% increase, while at the same time these two patients received similar cumulative air kerma radiation doses – 21.40 mGy and 27.10 mGy, respectively, – during fluoroscopy time. A statistically significant rise in the frequency of DRF was observed even in the first group of patients who received very small radiation doses. The absence of correlation between cumulative air kerma dose received by the patients from four dose groups and the frequency of DRF also supports the idea of inter-variability in radiosensitivity to low-doses of ionizing radiation.

The rise in radiation damage in blood cells after low dose irradiation of patients during vertebroplasty demonstrated in our study is also in agreement with the linear no threshold (LNT) model for cancer risk assessment. The consensus of regulatory radiation protection organizations for the calculation of the radiation-induced cancer risk is currently based on the LNT model, without a threshold safe dose [26-28].

The background frequency of DRF before vertebroplasty is significantly higher in all patients compared with the population control group. This could be due to CT examinations prior procedures and is in line with the results of Colin et al. who found an increased background number of γ -H2AX DNA damage-repair foci and an increased radiation-induced

number of γ -H2AX DRF in cell cultures of primary mammary epithelial cells of high-risk patients compared to low-risk patients [29].

Our study found no significant correlation between FPC frequency and age and cumulative air kerma dose. This absence of correlation could be due to limited age range of elderly patients and the wide variability in individual radiosensitivity. At the same time, we found tendency in increasing the cumulative air kerma dose received by the patient during fluoroscopy time with increasing BMI. To better understand this relation a wider group of patients should be further analyzed.

CONCLUSION

Our preliminary study showed a significant increase in DNA damage in patients after minimally invasive vertebroplasty procedures. These data can contribute to the future development of strategies for reducing the radiation dose to the patient where it is possible. Further investigation of the amount of miss-repaired DNA double-strand break radiation damage in cells is needed for evaluating the low dose and very low dose radiation risk for patients and medical personnel.

Ethics statement

All patients and controls included in this study were informed about the purpose of the study prior to participation and provided their written informed consent.

Conflict of interest

The authors of this study declare no conflict of interest.

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